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We summarize the new accomplishments that were made possible as a result of the acquisition of two CCD detectors (one gateable CCD detector and one liquid nitrogen cooled CCD detector, both from Princeton Instruments) with one common controller. Progress has been made jointly with Drs. Ronald Pinnick and Steven Hill (of the Army Research Laboratory, WSMR, NM) mainly in the area of fluorescence-spectra determination of single biological airborne particle as the particle flew by the pulsed UV laser focal volume. By using the gateable CCD detector, we have demonstrated the ability to capture the fluorescence spectrum on one type of particle while ignoring others. The decision as to what type of particles to capture the fluorescence spectrum is solely based on the particle fluorescence and/or elastic scattering characteristics. By using the liquid nitrogen cooled CCD detector, we were able to determine, with several UV laser wavelengths (266 nm and 355 nm), the fluorescence spectra of a large number of biological particles in various environments, i.e., whether the biological spores on a glass slide are dry or in an optical cell containing water. Spectral shifts and changes in the fluorescence intensity are noted. The usage of these DURIP-Yale instruments in other DoD sponsored research topics are briefly mentioned.

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"GATEABLE CCD DETECTOR FOR LASER SPECTROSCOPIC IDENTIFICATION OF AEROSOLS"

FINAL REPORT

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ABSTRACT

We summarize the new accomplishments that were made possible as a result of the acquisition of two CCD detectors (one gateable CCD detector and one liquid nitrogen cooled CCD detector, both from Princeton Instruments) with one common controller. Progress has been made jointly with Drs. Ronald Pinnick and Steven Hill (of the Army Research Laboratory, WSMR, NM) mainly in the area of fluorescence-spectra determination of single biological airborne particle as the particle flew by the pulsed UV laser focal volume. By using the gateable CCD detector, we have demonstrated the ability to capture the fluorescence spectrum on one type of particle while ignoring others. The decision as to what type of particles to capture the fluorescence spectrum is solely based on the particle fluorescence and/or elastic scattering characteristics. By using the liquid nitrogen cooled CCD detector, we were able to determine, with several UV laser wavelengths (266 nm and 355 nm), the fluorescence spectra of a large number of biological particles in various environments, i.e., whether the biological spores on a glass slide are dry or in an optical cell containing water. Spectral shifts and changes in the fluorescence intensity are noted. The usage of these DURIP-Yale instruments in other DoD sponsored research topics are briefly mentioned.

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INTRODUCTION

Real-time measurements of fluorescence spectra of airborne particles can be used for discrimination between biological and non-biological (such as sand) particles. The primary fluorescent compounds in most bacterial cells are amino acids (tryptophan, tyrosine, and phenylalanine, with excitation/emission wavelength in the range of 240-280/280-350 nm), reduced nicotinamide adenine dinucleotides (NADH, excitation/emission maximum around 340/450 nm), and flavin compounds (excitation/emission maximum 450/515-565 nm). Hence, by comparing the fluorescence spectra of an unknown airborne particle with these known biological compounds, a preliminary screening of the airborne particles into biological and non-biological types can be achieved. In addition, some crude form of biological species identification may also be possible, by using some form of "inversion" with the primary fluorescence compounds as the basis set.

Before the DURIP-Yale award, we and the ARL group borrowed, for a week, a gateable CCD detector from Princeton Instrument. We demonstrated, that as the airborne particles flew by the upper portion of a cw 488 nm laser beam ($\approx$ 300 μm in diameter), the two photomultiplier outputs (one to detect the elastic scattering intensity and another to detect the fluorescence intensity) could be used to form the decision whether or not to "gate on" a CCD detector. Once the CCD detector is gated on, it can examine the fluorescence spectrum of the *same* particle that flew by the lower portion of the laser focal volume. The time delay between traversal of the upper and lower portions of the laser beam is dependent on the particle velocity, which is determined by the sheath flow velocity of the inlet nozzle.

We labeled the above mentioned technique as **conditional sampling**[1], because we are sampling the fluorescence spectra conditioned on the two PMT outputs by some form of logic decisions, such as, AND, OR, or NAND. The only reason the cw 488 nm laser was selected, was because it was readily available. We were aware of the

shortcoming in using the cw 488 nm laser, because the 488 nm can only excite the fluorescence of flavins in biological samples. Nevertheless, we demonstrated we can indeed apply the conditional sampling technique to capture only the fluorescence spectra of Bacillus subtilis in a mixture of Bacillius subtilis and kaolin (some form of sand that is non-fluorescent). Thus, much time and computer memory can be saved by not having to store the blank fluorescence spectra of kaolin. Because we can selectively capture the fluorescence spectrum of a suspected particle, we can partially compensate for the slow read and storage time (about 100 Hz) of the CCD detector. Armed with the conditional sampling data, we petitioned in the DURIP proposal for a gateable CCD detector.

After receiving the approval from ARO (for $50,080) and Yale ($12,520) for a gateable CCD detector, we negotiated with Princeton Instrument for an educational discount. While keeping the total cost of $70,000, we were able to purchase two detectors, one gateable image-intensified CCD and the other a liquid nitrogen cooled CCD, which are controlled by one single controller. For single laser pulse circumstances, we should use the image-intensified CCD detector because of its gateable and amplification features. However, the use of the ICCD detector is at the expense of lower photoelectron quantum efficiency (photons to electrons) and shorter integration time, (because of its high dark current). For circumstances in which the data can be accumulated, we should use the liquid nitrogen cooled CCD detector, because of its much higher quantum efficiency and longer integration time associated with its much lower dark current.

RESEARCH ACCOMPLISHMENTS

Both the ICCD and CCD detectors were delivered by Princeton Instruments in November, 1995. After a month of "learning" how to use the ICCD detector, we set off to the ARL, located at White Sands Missile Range in New Mexico, in December, 1995 with these detectors to conduct another joint experiment. The results of this joint experiment are summarized under the heading of Flowing Particles: The Use of an ICCD Detector. After January 1996, we conducted several experiments at Yale, with the collaboration of our colleagues from ARL, using the CCD detector. The results of this joint experiment are summarized under the heading of Stationary Particles: The Use of a CCD detector.

Flowing Particles: The Use of an ICCD Detector

A pulse UV laser (266 nm), the fourth-harmonic of a flashlamp pumped Nd:YAG, was made to fire only if certain preset conditions from the two photomultiplers (PMT's) were met. That is, the laser firing is conditioned on the "logic" (OR, AND, or NAND) of the PMT signals arising from the elastic scattering and fluorescence excited by the beam from a cw argon-ion laser (488 nm). The two PMT's (one for the elastic scattering and the other for the fluorescence) and the cw argon-ion laser were part of the equipment used for the conditional sampling technique, mentioned in the introduction section.

In the new experiment, the argon-ion laser beam was aligned 4 mm below the tip of the inlet nozzle while the pulsed UV beam is focused 5 mm below the tip, or 1 mm below the argon-ion laser beam. The aerosols follow the sheath flow at a speed $\approx$ 20 m/s. If the logic conditions from the PMT's are satisfied, the delay time of 50 μs (1 mm path difference divided by 20 m/s) is sufficient to "trigger" the Q-switched Nd:YAG laser (equipped with two doubling crystals, first to the second harmonic at 532 nm and then the second harmonic of that, or 266 nm beam). This UV laser will fire provided that the flashlamp (running at 10 Hz) has occurred. This technique of firing the UV laser (266 nm), based on the two PMT outputs, is labeled as **conditional firing**.[2]

Once the UV laser is triggered to fire on the conditional firing mode, the image-intensified CCD detector (hereafter referred to as the ICCD detector) is gated on. The ICCD detector captures the UV-excited fluorescence spectrum of the particle, which during the time it was upstream in the argon-ion laser beam, has been considered by the two PMT's (based on the logic setting of the elastic and fluorescence outputs) as a particle that needs to be further examined by the pulsed UV laser beam. If the conditions were not met according to the preset logic conditions, then the particle will flow past the location of the UV laser beam focal volume, without triggering the UV laser and the gated ICCD detector. Thus, time, electrical power, and computer storage will not be wasted in capturing the fluorescent spectrum of an unsuspected particle, that may be in abundance relative to the suspected particles.

The upper limitation on the rate of suspected particles can not exceed the ICCD detector read and store rate, which is at present 10 Hz. Commercial grade ICCD detectors with faster rates are becoming more available. In our December 1995 conditional firing experiment (conducted at ARL), the upper limit on the rate of suspected particle was set by the flashlamp pumped Nd:YAG laser, which in our case was 10 Hz. However, this rate has already been increased to a few kHz with the advent of cw or pulsed semiconductor-diode pumped Nd:YAG laser and UV frequency doubling crystals with much larger nonlinear susceptibility and improved damage threshold.

Another severe limitation of our December 1995 experiment (conducted at ARL) was the use of the cw argon-ion laser (488 nm) as the "screening" laser to excite the elastic scattering and fluorescence. Because of the excitation wavelength (488 nm), only the flavin fluorescence could be excited. Thus, the conditional firing decisions could only have been made based on whether or not the airborne particle emitted fluorescence at 515-565 nm range. Only recently, Spectra Physics (Millennia) and Coherent (Verdi) have introduced two cw Nd:YAG lasers (both pumped by semiconductor laser diodes) that emit over 5 watts of green at 532 nm. It will be only a matter of time when these green lasers

can be further doubled in frequency, to produce at least one watt of 266 nm. By using a cw 266 nm diode-pumped Nd:YAG laser, instead of the 488 nm argon-ion laser that we had used, will then enable us to make the conditional firing decisions based on whether or not the airborne particles emitted fluorescence in the wavelength range (280-350 nm) that is characteristic for amino acids.

We used outdated lasers (argon-ion and flashlamp pumped Nd:YAG) as the cw interrogation beam to form the logic decisions to trigger the pulsed high-energy UV beam at 266 nm, which can excite enough fluorescence in order to detect its spectrum. Nevertheless, we were able to demonstrate the potential of conditional firing in terms of selectively triggering the high-energy UV laser and gating on the ICCD detector only when the suspected particle flew past the high-energy UV laser focal volume. We will summarize some of these results, that used the outdated lasers but the state-of-the-art ICCD detectors purchased with the DURIP-Yale funds. For details, please see reference 2, which will soon appear in print in Optics Letters. A preprint has been submitted to ARO (via Battelle).

One of the conditional firing features is that the pulsed UV laser fires only when there is a suspected particle in its laser focal volume. Figures 1(a) and (b) shows 10 consecutive spectra recorded in the laser free-running mode and in the conditional firing mode, respectively. Figure 1 (a) shows that in the free-running mode, every time the pulsed laser is fired (for 10 ns), there is no particle in the laser focal volume. However, when one of the PMT is "on" because of the elastic scattering of the particle traversing the argon-ion laser focal volume, the conditional fired pulsed UV laser will always find a particle in its focal volume. Figure 1 (b) shows that every time the UV laser fires and the ICCD detector is gated on, there is a particle in the UV laser focal volume and the ICCD captures the fluorescence spectrum for each laser firing.

Another feature of the conditional firing technique is to fire the pulsed UV laser only when the two PMT outputs satisfy certain preset logic conditions. Figures 2(a) shows

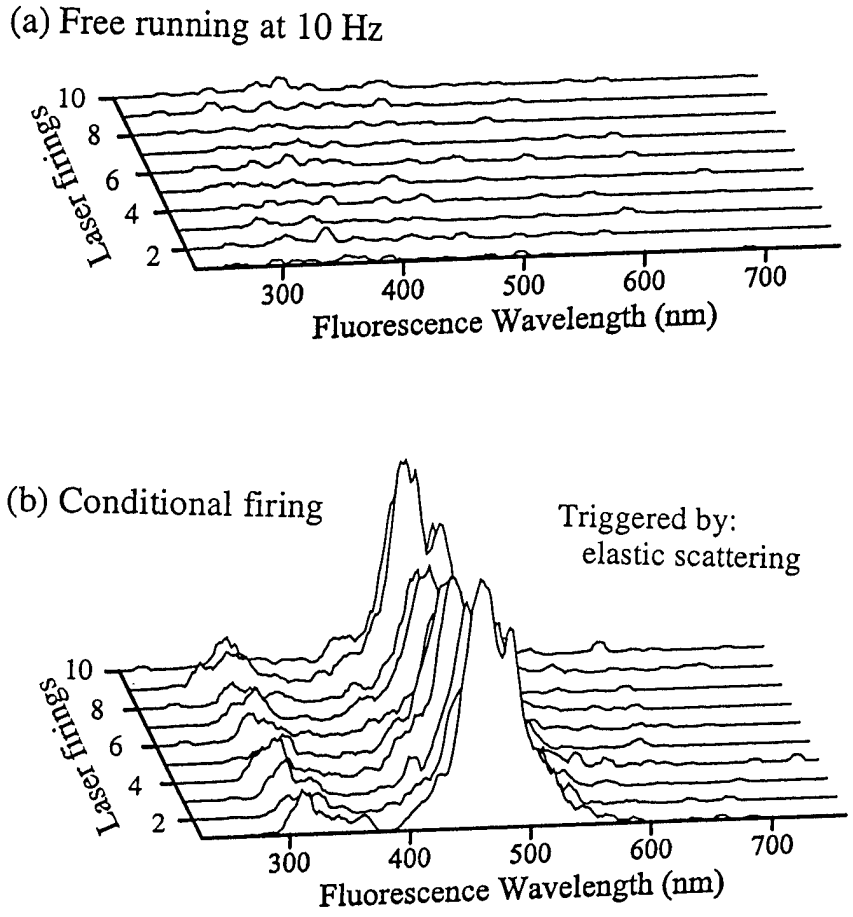

Figure 1. To detect the fluorescence of blue-dyed polystrene spheres, twenty consecutive ICCD recordings are taken, with the system in a free running mode (a) and in a conditional firing mode (b). The latter is conditioned on the 488-nm elastic scattering

the case when there is a mixture of green and red dyed polystyrene spheres, both with 3 μm diameter. The logic is set for the condition that **only** the elastic scattering sensing PMT needs to be "on" for the UV laser and the ICCD detector to trigger. For this logic setting, the resultant fluorescence spectra for each UV laser firing is either from the red or from the green particle. The spectral peak at 320 nm is the intrinsic fluorescence from polystyrene itself, present regardless of any added dye.

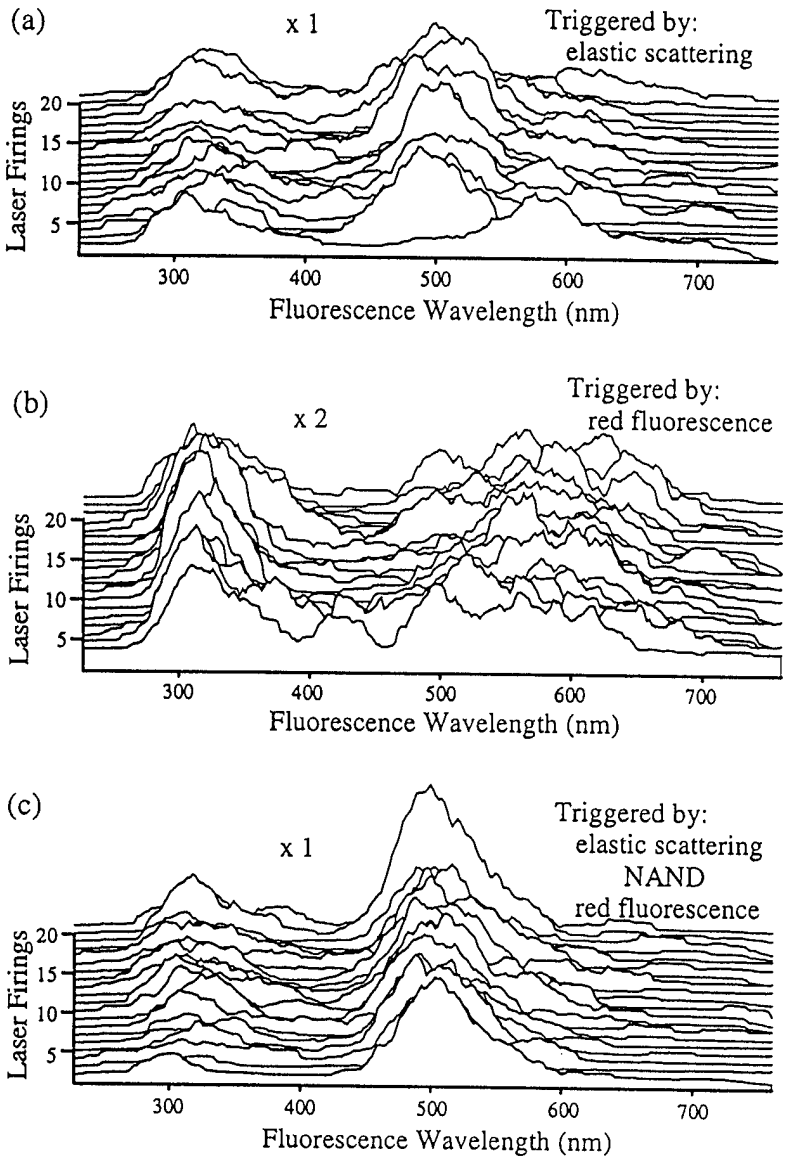

Figure 2. Three conditional logic cases set according to the two PMTs voltage thresholds. (a) when only the elastic scattering signal is used, both green- and red-fluorescence spectra are recorded; (b) when only the red-fluorescence signal is used, the fluorescence spectra from the red-dyed spheres alone are recorded; and (c) when the elastic scattering NAND red fluorescence signals are used, only the fluorescence spectra from the green-dyed spheres are recorded.

By selecting the logic such that the red-fluorescence PMT must be on before the pulsed UV laser and the ICCD detector are turned on, then only when the red polystyrene is inside the UV laser focal volume would the red-particle fluorescence spectrum be captured. Figure 2(b) shows that every time the ICCD detector is on, only the fluorescence spectrum of the red polystyrene particles are detected, even though there is nearly an equal

mixture of red and green polystyrene spheres (both of the same diameter) flow past the cw Argon-ion laser or screening laser beam.

By selecting the logic for the two PMT's in the state of elastic scattering NAND red fluorescence, the pulsed UV laser and ICCD detector are turned on only when the elastic scattering sensing PMT **and not** the red fluorescence sensing PMT is on. Figure 2(c) shows that every time the ICCD detector is on, only the fluorescence spectrum of the green polystyrene is detected, even though there is nearly an equal mixture of red and green polystyrene spheres of the same 3 μm diameter.

The usage of the cw 488 nm argon-ion laser as the screening laser beam and the red and green polystyrene spheres of 3 μm diameter as the sample is intended to illustrate that particle selection based on elastic scattering and fluorescence (within a wavelength band) intensities is possible. Had we been able to use a cw 266 nm Nd:YAG laser as the screening laser to excite the fluorescence intensity of amino acid, NADH, flavin, or chlorophyll compounds within a given airboune particle, then we could have selected which airborne particles to detect its florescence spectrum with the pulsed UV laser and ICCD detector. For example, if we had selected the NAND logic condition for the elastic-scattering sensing PMT and the red-fluorescence sensing PMT (for the chlorophyll), the pulsed UV laser and the ICCD detector would only turn on when the non-leaf particles (containing no chlorophyll) are traversing the pulsed UV laser focal volume. Alternatively, if we had selected the AND logic condition for the elastic scattering sensing PMT and the blue fluorescence sensing PMT (for the amino acids), the pulsed UV laser and the ICCD detector would only turn on when the biological particles were traversing the pulsed UV laser volume. In principle, even more sophisticated logic settings can be selected, for example depending on the fluorescence intensity ratio between the amino acid band and the NADH band. Suffice it to say that we have, by using polystyrene spheres, demonstrated the ability to do some presorting of the airborne particles before its fluorescence spectrum is examined and recorded by the ICCD detector.

The ICCD detector can also be used to capture the fluorescence spectra of biological airborne particles, excited by the pulsed UV laser at 266 nm. In December 1995, the ARL-Yale group recorded the fluorescence spectra [shown in Fig. 3(a)] of nebulized dried airborne particles of tyrosine, tryptophan, NADH, and riboflavin. Each spectrum is the accumulation of 100 laser firings with the logic set to trigger whenever the elastic scattering sensing PMT is on. In addition, airborne spores (of unknown cluster dimensions) of Bacillus subtitles and Bacillus thuringiensis are detected and Fig. 3(b) shows their fluorescence spectra along with that of tryptophan. The spectral lineshape for wavelength below 290 nm is distorted by the attenuation of the blocking filter which must be used to diminish the elastic scattering at 266 nm. In particular, the tyrosine spectrum is highly distorted because the block filter (WG 305 nm) is used, which has a 30 nm transition range (from 5% to 80% transmission) centerd at 280 nm.

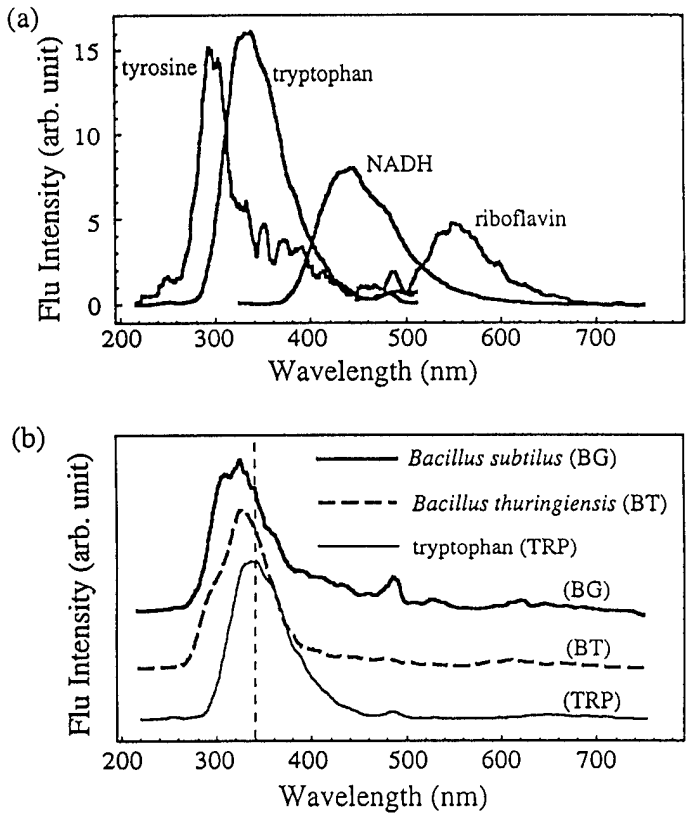

Figure 3. Accumulation of 100 conditional-firing UV-laser excited fluorescence spectra from biological compounds (a) and bacterial particles (b). Conditional firing is based on the 488-nm elastic scattering. In (a), the short wavelength side of the tyrosine lineshape is affected by the transmission of the long-pass optical filter.

Stationary Particles: The Use of a CCD detector

Whenever spectral data can be accumulated in time, i.e., non-single event data, the CCD detector should be used instead of the ICCD detector, because of the CCD detector's much higher quantum efficiency and lower dark noise. After January, 1996, we started to plan to record fluorescence spectra of a variety of biological particles with 266 nm and 355 nm incident radiation. The ARL collaborators loaned us the second doubling crystal to covert the 532 nm radiation (second-harmonic of the Nd:YAG laser) into 266 nm. We already had a sum-frequency crystal to combine the 532 nm and the 1064 nm (primary beam) into 355 nm.

The key objective of this phase of our program is to build up a library of fluorescence spectra from a variety of biological particles when they are "wet" and "dry". The samples are either dissolved or suspended in water and placed in an optical cell (in the figures the curves are labeled as "wet") or placed in a solid-sample holder in the dry state (in the figures the curves are labeled as "dry"). We wanted to use the "same" spectral dispersion (spectrograph) and CCD detector system for all the samples in the wet and dry condition. Furthermore, we wanted to use a better 266 nm laser block filter, a liquid *N,N*-Dimethylfomamide (abbreviated as DMF) placed in a 1 cm thick quartz optical cell. The DMF used as a 266 nm laser blocking filter has a much steeper "step-function" transmission curve then the WG-305 glass filter used in our December 1995 experiment. For a 1 cm cell length, transmission at 266 nm is <1.5%, the 50% transmission wavelength occurs at 275 nm, and the 80% transmission occurs 290 nm.

Figures 4 shows the fluorescence spectra (excited by 266 nm) of phenylalanine, tyrosine, tryptophan and riboflavin (in their wet and dry states). Figures 5 shows the fluorescence spectra (excited by 266 nm) of bacillus subtilus, bacillus thurigiensis, albumin and casein (in their wet and dry states). Because of the use of DMS as a 266 nm blocking filter, the spectral shape of tyrosine is now more extended to the blue, compared to that

shown in Fig. 3(a). Note that the spectral shifts of the same biological species in the wet and dry states do not all shift to the red or to the blue. The secondary peak (at ≈ 400 nm) of tyrosine is particularly sensitive to the change in its environment, i.e., whether the sample is wet or dry. The fact that the fluorescence peak of NADH overlaps with the secondary peak of tryptophan could lead to complications, particularly if a band-pass filter centered at 400 nm is used to detect the presence or absence of NADH. The subtle changes in spectral lineshape attest to the need to measure the fluorescence spectrum of airborne particle with a spectrograph and CCD or ICCD detector rather then the fluorescence intensity within an spectral range with a band-pass filter and a PMT.

Figures 6 compares the fluorescence spectra (excited by 355 nm and 266 nm laser with the same excitation energy and detection efficiency) of NADH, riboflavin, *bacillus subtilus* and *bacillus thuringiensis*. With 355 nm as the excitation wavelength, the fluorescence of tyrosine and tryptophan are not induced. Therefore, the fluorescence peak at 450 nm with 355 nm excitation can be assigned to NADH, rather than a combination of NADH and tyrosine's secondary peak (as in the case with 266 nm excitation). In addition, 355 nm excitatin is more efficient for exciting the fluorescence of NADH and riboflavin. Consequently, the use of 355 nm as an excitation source, in addition to 266 nm as an excitation source, may prove to be useful in further discriminating amongst biological airborne particles.

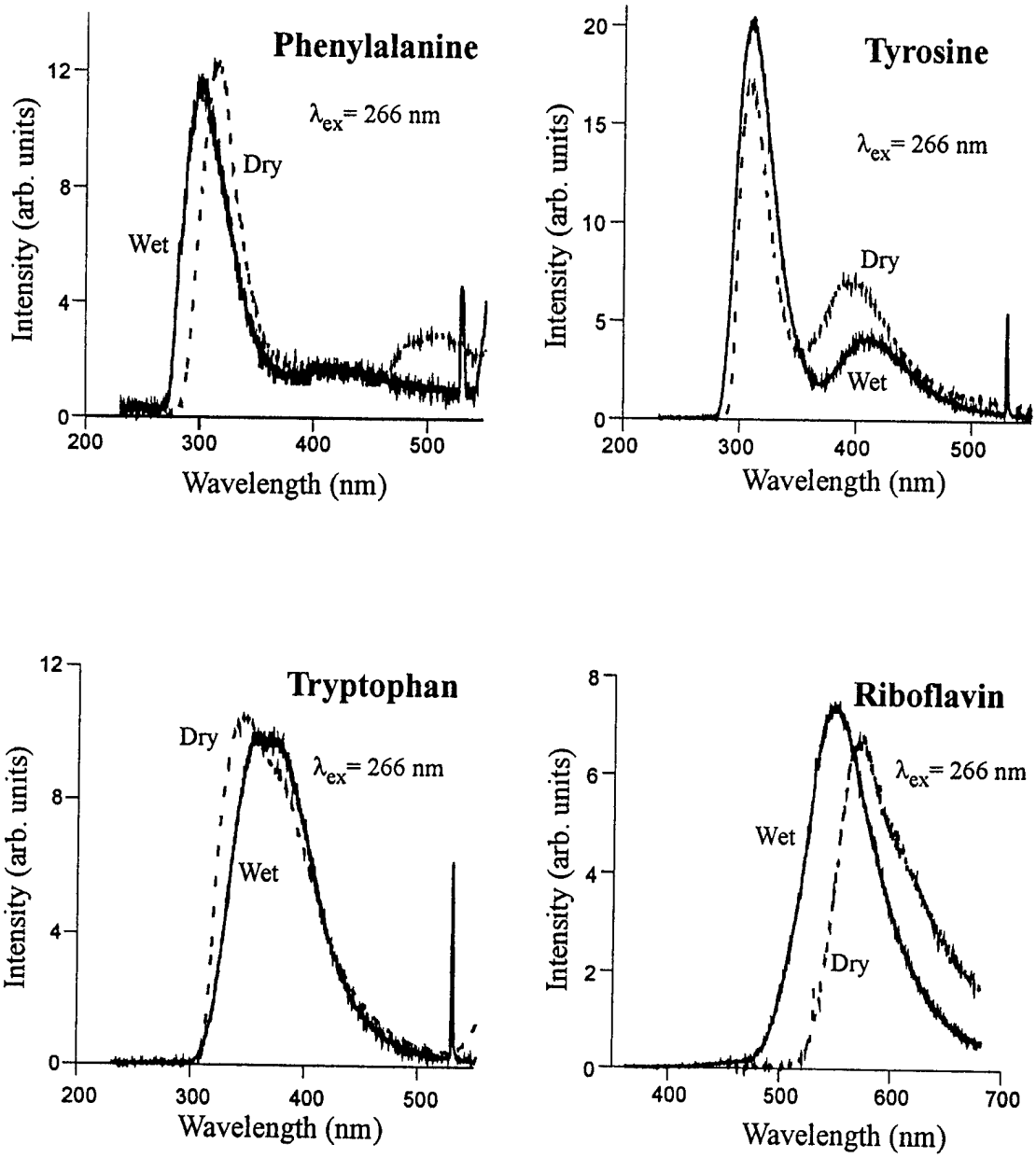

Figure 4. Fluorescence spectra of phenylalanine, tyrosine, tryptophane and riboflavin in a dry and a wet (aqueous solution) envoronment. The excitation wavelength is at 266 nm.

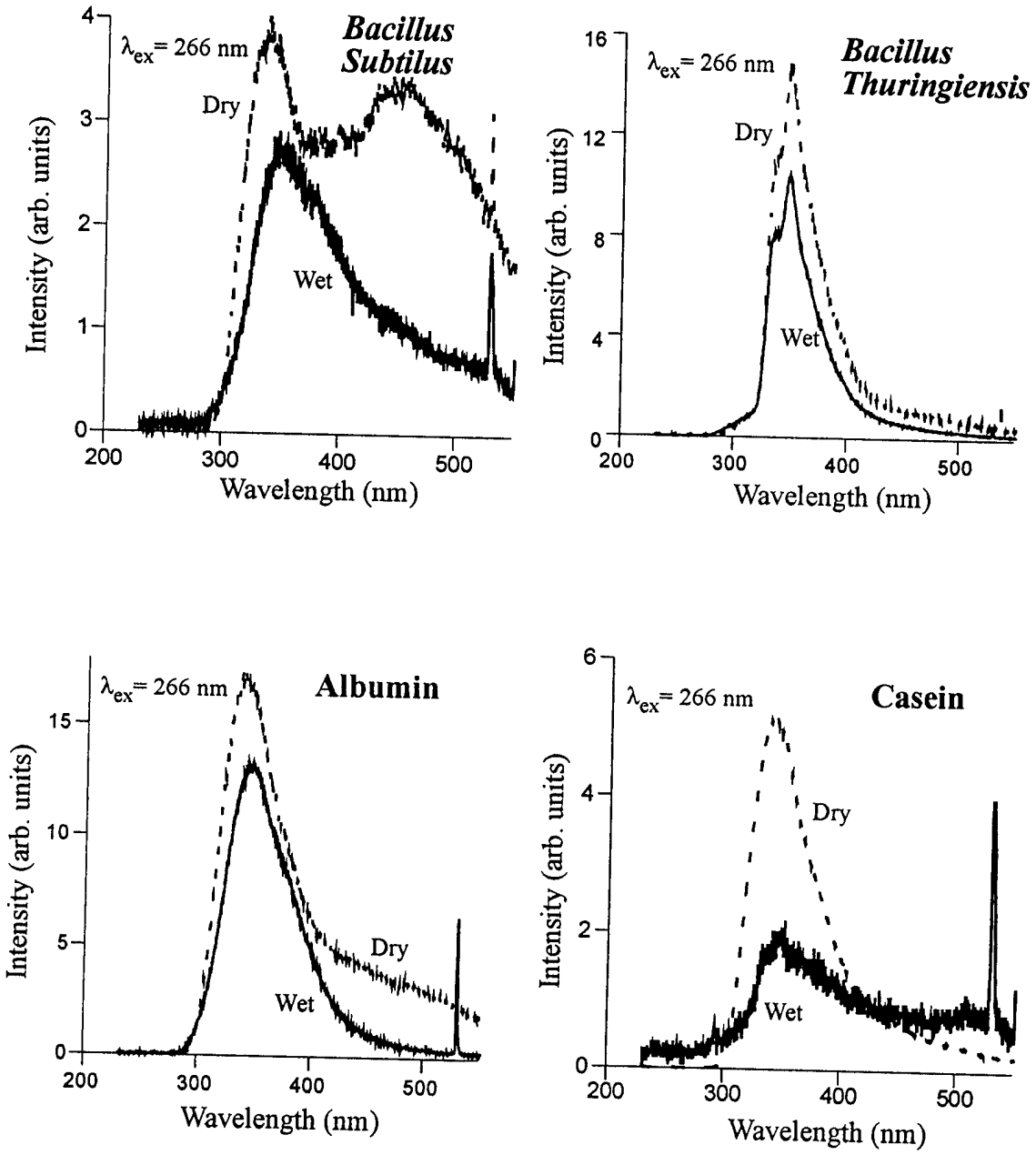

Figure 5. Fluorescence spectra of BG, BT, bovine albumin, and casein in a dry and a wet (aqueous solution or suspension) environment. The excitation wavelength is at 266 nm.

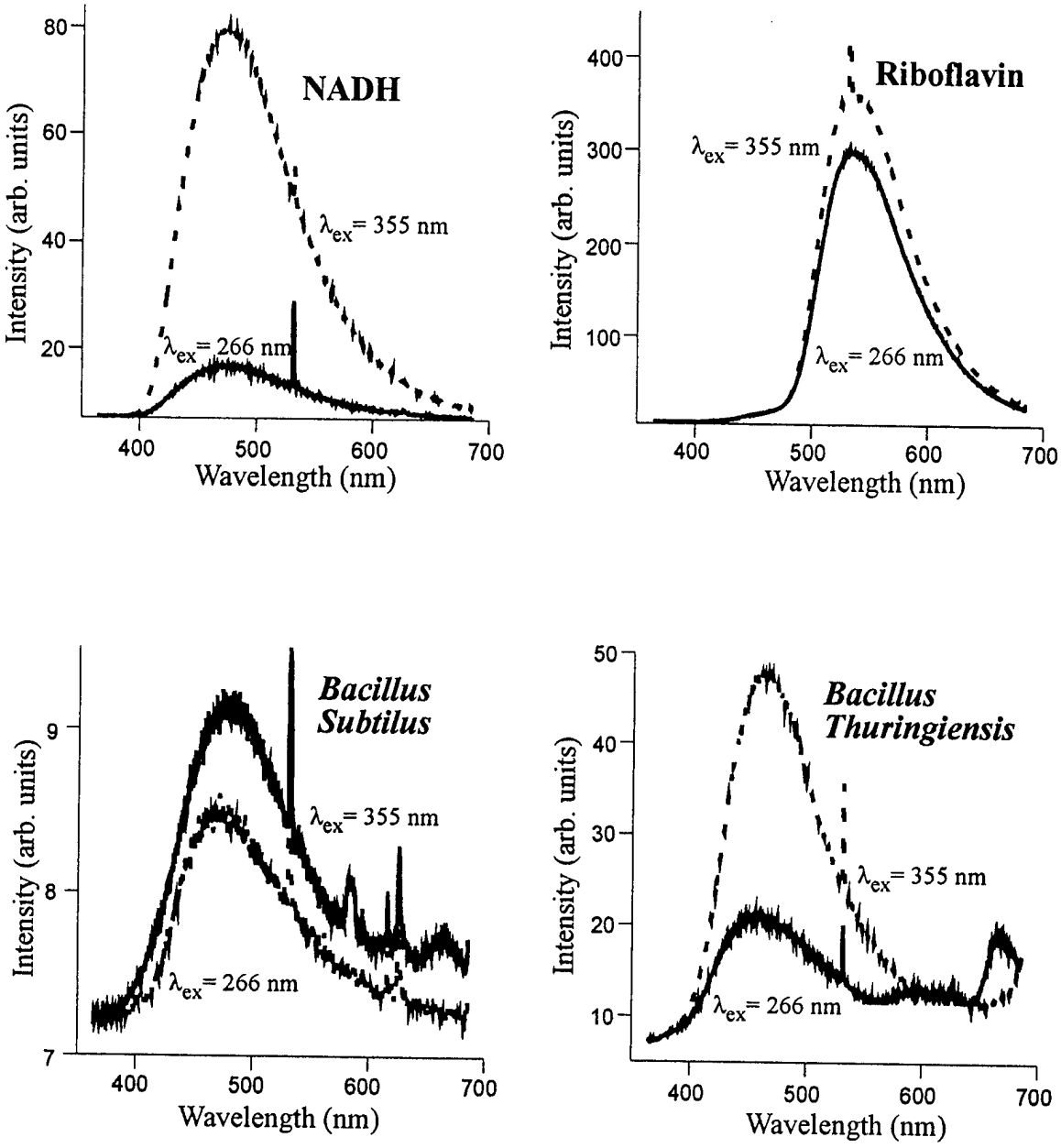

Figure 6. Comparison of fluorescence spectra of NADH, riboflavin, BG, and BT when excited with 266 nm and 355 nm wavelengths respectively. The pump energy for 266 nm and 355 nm wavelengths is kept the same in each measurement.

The Usage of DURIP Equipment in other DoD Projects

The availability of the ICCD and CCD detector has potential benefits to the two existing DoD grants, AFOSR (F49620-99-0135) and ARO (DAAH04-94-G-0031). Their potential benefits are briefly mentioned.

In the AFOSR grant, we are currently studying the effect of external seeding a micrometer-sized droplet with radiation at the Stokes-shifted Raman wavelength of minority species, in order that the stimulated Raman scattering (SRS) signal from a minority species will "grow faster" than the SRS signal of the majority species.[3,4] At present a linear array of intensified photodiode array is used. This one-dimensional detector will be replaced by the two-dimensional ICCD detector when we wish to compare the SRS signal from a multiple of droplets, that are imaged along the slit of the spectrograph. In so doing, we can capture the SRS signal from many droplets, not just a single droplets as we are presently investigating.

In the ARO grant, we are currently studying the effect of surfactant on a hanging "pendent" water droplet. The surfactants are chosen to have both fluorescence and a large second-harmonic generation susceptibility coefficient. At present, we are measuring both the fluorescence image and second-harmonic-generation image. In particular, we are studying their polarization dependence as a function of the "packing density" of the surfactant when the pendent droplet size is changed. In the less dense packing state, the orientation of the surfactant is more random. However, when the packing density is increased, the surfactant molecules are oriented more radially. At present, we are using an ordinary room temperature video camera to record the fluorescence image of the pendent droplet. However, we require many orders of improvement in the detector sensitivity in order to capture the second-harmonic image. Our plans are to use the liquid nitrogen cooled CCD detector to capture the second-harmonic image of the hanging pendent droplet for various droplet size and, hence, surface packing density.

The availability of the ICCD and CCD detector, even though there is but one controller that needs to be shared between these two state-of-the-art detectors, is a tremendous benefit to the present and future-planned AFOSR and ARO projects. We are grateful to ARO for awarding the DURIP proposal, Yale University for cost-sharing the DURIP budget, the two scientists (Drs. Ronald Pinnick and Steve Hill) at ARL for their collaboration in every sense of the word.

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Research Assoc.

Xiaoyun Pu

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Mohiuddin Mazumder

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Mitchell Fields

Seongsik Chang

Thomas Heldt

Andrew Poon

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4. Mitchell H. Fields, Jürgen Popp, and Richard K. Chang, "External Seeding of Stimulated Raman Scattering in Microdroplets," Optics Letters (to be published).

FINANCIAL SUMMARY

The items we actually purchased differ in detail to what we initially petitioned in DURIP. Princeton Instruments, through their educational discount, was able to sell us something better than what we petitioned in the DURIP proposal. The details are as follows:

What Was Petitioned in the DURIP Proposal.

Lens Coupled Intensified Liquid Nitrogen Cooled CCD Detector System (consisitng of):

a) LCI/2.3 Lens coupled intensifier
b) LN/CCD-1100PB/UVAR CCD Dectector
c) ST-130 DMA/CCD Controller/Interface to AT Computer (or comparible)
d) PG-200 Digital Gate Pulse Generator
e) WinSpec Software for IBM/AT Computer (or comparible).

Total $62,600

Total requested from DURIP	$50,080
Matching fund from Yale University (20%)	$12,520

What Was Actually Purchased with the DURIP-Yale Funding

Gateable Intensified CCD Optical Detection System. See attached quote, includes:

a) ICCD-1024 MLDS Detector

b) ST-138 High performance CCD camera controller for AT and MacIntoch computers.

c) PG-200 Digital Gate Pulse Generator

d) WinSpec Software for IBM/AT computer, or compatable

e) All necessary software and hardware

f) Detector Adaptor for Spectrometer (Acton, SpectraPro 500mm)

PLUS: included as special purchase with System 1 above:

g) LN/CCD-1100PB/UVAR CCD Detector

Total Cost: $70,000